Pak. J. Biotechnol. Vol. 11(2) 111-121
www.pjb.org

ISSN Print: 1812-1837
ISSN Online: 2312-7791

MOLECULAR DIVERSITY IN SOME A-GENOME WHEAT AMPHIPLOIDS (2n=6x=42; BBAAAA)

Sania Ahmed[1], Hadi Bux[2*], Alvina Gul-Kazi[3], Abdul Wajid Channa[2], Sadaf Tabasum Qureshi[2], Aijaz Ahmed Soomro[4],Mahboob Ali Sial[5], Abdul Rauf[1] and Abdul Mujeeb-Kazi[6]

[1]PMAS-Arid Agriculture University Rawalpindi, Pakistan.[2]Institute of Plant Sciences, University of Sindh Jamshoro, Pakistan.[3]Atta-ur-Rehman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad, Pakistan.[4]Department of Agronomy, Faculty of Crop Production, Sindh Agriculture University, Tandojam, Paksitan.[5]Nuclear Institute of Agriculture, Tandojam, Pakistan, [6]National Institute of Biotechnology and Genetic Engineering Faisalabad, Pakistan
Email*: hadiqau@gmail.com

Article received September 15, 2014; Revised September 25, 2014; Accepted October 2, 2014

ABSTRACT

Wild relatives of wheat have defying capability against detrimental conditions as they possess rich reservoirs of valuable genes. Through hybridization, many desired traits have been successfully introgressed from wild relatives to cultivars for various stress tolerances for wheat improvement. Wheat amphiploids (BBAAAA) have been created from diploid resources (*Triticum monococum* (AA), *Triticucm urartu* (AA), and *Triticum bioeticum* (AA) and *Triticum turgidum* the tetraploid durum (BBAA) wheat cultivars through bridge crossing. These amphiploids possess enormous variability for biotic and abiotic stresses. In current study, molecular characterization of a collection of 79 amphiploids (2n=6x=42, BBAAA) by 25 SSR primers have been carried out. The molecular scanning produced 58 polymorphic bands and all were polymorphic showing 100% polymorphism. Dendrogram based on Nei and Li's similarity coefficient, clearly distinguished the genotypes in the clusters showing abundant diversity. The genetically diverse germplasm identified through genetic similarity and cluster analysis in current study are accession 13, 16, 42, 52 and 50. These amphiploids received the A genome from diploid *Triticum bioeticum*. The selected collection should be used for the genetic improvement of wheat and the selected collection needs further studies to reveal the hidden desirable variability of agricultural utility.

Key words: SSRs, genetic and molecular diversity, synthetic wheats, amphiploids, cluster analysis, polymorphic loci.

INTRODUCTION

For optimal conservation of germplasm from genetic erosion, the studies on population structure and genetic variability of wheat are pre-requisite. Modern cultivated wheat genotypes are deficit in genetic diversity which is necessary for conservation of genetic resources from erosion (Sofalian *et*

al., 2008; Safdar *et al*., 2013). Whenever new alleles are required and genetic base becomes narrow then necessity is felt to incorporate such novel diversity from family resources that has broad genetic resources. An essential intermediate step is creation of stable amphiploids by which required genes can be transferd from related wild species to wheat crop.Genetic diversity analysis can be used based on morphological, pedigree, molecular (DNA based) and biochemical and agronomic performance data in individuals (Mohammadi and Prasanna, 2003). Morphological data based genetic diversity got suffered from drawback that is effected by environment and traits are numerically limited (Maric *et al*., 2004). However, molecular markers do not need previous information of pedigree and not affected by environment as they are direct gene products (Jefferies *et al*., 1999; Bohn *et al.*, 1999).

The genetic markers are employed for genetic evaluation, among them most prominent, effective, authentic to differentiate nearly related culti-vars; precise are the molecular markers (Saleh et al., 2012). Among molecular markers, simple sequence repeats (SSR) are most appropriate type having capability to discriminate or identify genotype within a species. DNA-based molecular markers are powerful tools used for gene mapping, DNA fingerprinting, and the genetic diversity-assessment in cereal crops (Figuiredo, 2013). Simple sequence repeats (SSRs) are one of most used genetic markers in wheat (Cook *et al*., 2004) because of their distribution throughout genome, excess informativeness, advantage of analysis by PCR and high polymorphism characteristics (Gupta and Varshney, 2000, Gupta *et al*., 1996). Microsatellite or (SSR) markers are the DNA fragments containing tendem repeats of short sequence (2-6 nucleotides) easily transferable between genotypes. The approach called-marker-assisted selection (MAS) has largely facilitated the swift selection of the genetic stocks carrying desirable traits. Wheat amphiploids (BBAAAA) have been created from diploid resources (*Triticum monococum* (AA), *Triticucm urartu* (AA), and *Triticum bioeticum* (AA) and *Triticum turgidum* the tetraploid durum (BB AA) wheat cultivars through bridge crossing (Gill et al., 1988; Ma *et al*., 1997). These genetic stocks have greater genetic variability and can be exploited for minor and major genes for tolerance to biotic and abiotic stresses (Kilian et al. 2011; Ahmed *et al*., 2014).The present study was aimed at the characterization of wheat amphiploids (BBAAAA) through SSR markers to identify genetically diverse genotypes for utilization in wheat improvement efforts.

MATERIALS AND METHODS

Wheat germplasm: A group of 79 wheat amphiploids (2n=6x=42; BBA AAA) were collected from wheat wide crosses and cytogenetics laboratory, National Agriculture Research Centre, Islamabad for molecular evaluation

(Table-1). The production protocol of by Mujeeb-Kazi (2006). these amphiploids has been reported

Table 1 Pedigree, genome of wheat amphiploids used for molecular analysis

Entry No	Pedigree	Genome	A Genome Donor	Durum Parent
1	YUK/T.BOEOTICUM (1) CIGM90.769	AABBAA	*T. boeoticum*	*T. turgidum*
2	YUK/T.BOEOTICUM(2) CIGM90.770	AABBAA	*T. boeoticum*	*T. turgidum*
3	STY-US/CELTA//PALS/3/SRN_5/4/ T.BOEOTICUM(3) CIGM90.640	AABBAA	*T. boeoticum*	*T. turgidum*
4	SCA/ T.BOEOTICUM(3) CIGM90.667	AABBAA	*T. boeoticum*	*T. turgidum*
5	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T.BOEOTICUM (3) CIGM90.771	AABBAA	*T. boeoticum*	*T. turgidum*
6	SCA/ T.BOEOTICUM(10) CIGM90.669	AABBAA	*T. boeoticum*	*T. turgidum*
7	GARZA/BOY// T.BOEOTICUM(10) CIGM90.773	AABBAA	*T. boeoticum*	*T. turgidum*
8	GARZA/BOY// T.BOEOTICUM(12) CIGM90.774	AABBAA	*T. boeoticum*	*T. turgidum*
9	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/ T.BOEOTICUM(13) CIGM90.775	AABBAA	*T. boeoticum*	*T. turgidum*
10	SCA/ T.BOEOTICUM (14) CIGM90. 671	AABBAA	*T. boeoticum*	*T. turgidum*
11	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T.BOEOTICUM(14) CIGM90.776	AABBAA	*T. boeoticum*	*T. turgidum*
12	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T.BOEOTICUM(15) CIGM90.777	AABBAA	*T. boeoticum*	*T. turgidum*
13	GARZA/BOY//T.BOEOTICUM(16)CIGM90.778	AABBAA	*T. boeoticum*	*T. turgidum*
14	BOTNO/ T.BOEOTICUM(20) CIGM92.440	AABBAA	*T. boeoticum*	*T. turgidum*
15	GARZA/BOY// T.BOEOTICUM(21) CIGM90.780	AABBAA	*T. boeoticum*	*T. turgidum*
16	SCA/ T.BOEOTICUM(23) CIGM90.674	AABBAA	*T. boeoticum*	*T. turgidum*
17	DOY1/ T.BOEOTICUM(23) CIGM90.781	AABBAA	*T. boeoticum*	*T. turgidum*
18	SHAG_22/ T.BOEOTICUM(24) CIGM92.1593	AABBAA	*T. boeoticum*	*T. turgidum*
19	DOY1/ T.BOEOTICUM(26) CIGM90.782	AABBAA	*T. boeoticum*	*T. turgidum*
20	DOY1/ T.BOEOTICUM(27) CIGM90.783	AABBAA	*T. boeoticum*	*T. turgidum*
21	SCA// T.BOEOTICUM(28) CIGM90.675	AABBAA	*T. boeoticum*	*T. turgidum*
22	DOY1/ T.BOEOTICUM(28) CIGM90.784	AABBAA	*T. boeoticum*	*T. turgidum*
23	SCA/ T.BOEOTICUM(31) CIGM90.676	AABBAA	*T. boeoticum*	*T. turgidum*
24	SCA/ T.BOEOTICUM(33) CIGM90.677	AABBAA	*T. boeoticum*	*T. turgidum*
25	SCOOP_1/ T.BOEOTICUM(33) CIGM90.V697	AABBAA	*T. boeoticum*	*T. turgidum*
26	SCA/ T.BOEOTICUM(34) CIGM90.678	AABBAA	*T. boeoticum*	*T. turgidum*
27	BOTNO/ T.BOEOTICUM(35) CIGM92.443	AABBAA	*T. boeoticum*	*T. turgidum*
28	D67.2/P66.270// T.BOEOTICUM(35) CIGM92.450	AABBAA	*T. boeoticum*	*T. turgidum*
29	SCA/ T.BOEOTICUM(36) CIGM90.679	AABBAA	*T. boeoticum*	*T. turgidum*
30	SCA/ T.BOEOTICUM(39) CIGM90.681	AABBAA	*T. boeoticum*	*T. turgidum*
31	SCA/ T.BOEOTICUM(40) CIGM90.681	AABBAA	*T. boeoticum*	*T. turgidum*
32	SCOOP_1/ T.BOEOTICUM(40) CIGM90.698	AABBAA	*T. boeoticum*	*T. turgidum*
33	SCOOP_1/ T.BOEOTICUM(46) CIGM90.699	AABBAA	*T. boeoticum*	*T. turgidum*
34	SCOOP_1/ T.BOEOTICUM(50) CIGM90.700	AABBAA	*T. boeoticum*	*T. turgidum*
35	LCK59.61/ T.BOEOTICUM(52) CIGM92.438	AABBAA	*T. boeoticum*	*T. turgidum*
36	STY-US/CELTA//PALS/3/SRN_5/4/ T.BOEOTICUM(54) CIGM90.642	AABBAA	*T. boeoticum*	*T. turgidum*
37	SHAG_22/ T.BOEOTICUM(55) CIGM92.1598	AABBAA	*T. boeoticum*	*T. turgidum*
38	AJAIA/ T.BOEOTICUM(55) CIGM92.1599	AABBAA	*T. boeoticum*	*T. turgidum*
39	AJAIA/ T.BOEOTICUM(56) CIGM92.1601	AABBAA	*T. boeoticum*	*T. turgidum*
40	SHAG_22/ T.BOEOTICUM(56) CIGM92.1600	AABBAA	*T. boeoticum*	*T. turgidum*
41	SCOOP_1/ T.BOEOTICUM(59) CIGM90.701	AABBAA	*T. boeoticum*	*T. turgidum*

42	SCOOP_1/ T.BOEOTICUM(60) CIGM90.702	AABBAA	*T. boeoticum*	*T. turgidum*
43	68.111/RGB-U//WARD/3/ T.BOEOTICUM(61) CIGM90.790	AABBAA	*T. boeoticum*	*T. turgidum*
44	SHAG_22/ T.BOEOTICUM(68) CIGM92.1602	AABBAA	*T. boeoticum*	*T. turgidum*
45	SCOOP_1/ T.BOEOTICUM(69) CIGM90.703	AABBAA	*T. boeoticum*	*T. turgidum*
46	SHAG_22/ T.BOEOTICUM(70) CIGM92.1603	AABBAA	*T. boeoticum*	*T. turgidum*
47	SCOOP_1/ T.BOEOTICUM(71) CIGM90.704	AABBAA	*T. boeoticum*	*T. turgidum*
48	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T.BOEOTICUM(74) CIGM92.455	AABBAA	*T. boeoticum*	*T. turgidum*
49	BOTNO/ T.BOEOTICUM(75) CIGM92.446	AABBAA	*T. boeoticum*	*T. turgidum*
50	D67.2/P66.270// T.BOEOTICUM(75) CIGM92.452	AABBAA	*T. boeoticum*	*T. turgidum*
51	SCOOP_1/ T.BOEOTICUM(79) CIGM90.705	AABBAA	*T. boeoticum*	*T. turgidum*
52	SCOOP_1/ T.BOEOTICUM(80) CIGM90.706	AABBAA	*T. boeoticum*	*T. turgidum*
53	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T.BOEOTICUM(83) CIGM92.456	AABBAA	*T. boeoticum*	*T. turgidum*
54	SCOOP_1/ T.BOEOTICUM(87) CIGM90.707	AABBAA	*T. boeoticum*	*T. turgidum*
55	SHAG_22/ T.BOEOTICUM(88) CIGM92.1605	AABBAA	*T. boeoticum*	*T. turgidum*
56	SCOOP_1/ T.BOEOTICUM(89) CIGM90.708	AABBAA	*T. boeoticum*	*T. turgidum*
57	SCOOP_1/ T.BOEOTICUM(90) CIGM90.709	AABBAA	*T. boeoticum*	*T. turgidum*
58	SCOOP_1/ T.BOEOTICUM(91) CIGM90.710	AABBAA	*T. boeoticum*	*T. turgidum*
59	SCOOP_1/ T.MONOCOCCUM(98) CIGM90.711	AABBAA	*T. monococcum*	*T. turgidum*
60	AOS/ T.MONOCOCCUM(98) CIGM90.791	AABBAA	*T. monococcum*	*T. turgidum*
61	AOS/ T.MONOCOCCUM(111) CIGM90.793	AABBAA	*T. monococcum*	*T. turgidum*
62	68.111/RGB-U//WARD/3/ T.MONOCOCCUM(112) CIGM92.463	AABBAA	*T. monococcum*	*T. turgidum*
63	BOTNO/ T.MONOCOCCUM (112) CIGM92.465	AABBAA	*T. monococcum*	*T. turgidum*
64	SCOOP_1/ T.MONOCOCCUM (118) CIGM90.712	AABBAA	*T. monococcum*	*T. turgidum*
65	AOS / T.MONOCOCCUM (118) CIGM90.794	AABBAA	*T. monococcum*	*T. turgidum*
66	FGO/USA2111// T.MONOCOCCUM (119) CIGM90.795	AABBAA	*T. monococcum*	*T. turgidum*
67	FGO/USA2111// T.MONOCOCCUM (122) CIGM90.796	AABBAA	*T. monococcum*	*T. turgidum*
68	DOY1/ T.URARTU (542) CIGM90.567	AABBAA	*T. urartu*	*T. turgidum*
69	DOY1/ T. URARTU (543) CIGM90.568	AABBAA	*T. urartu*	*T. turgidum*
70	DOY1/ T. URARTU (550) CIGM90.570	AABBAA	*T. urartu*	*T. turgidum*
71	68.111/RGB-U//WARD/3/ T. URARTU (550)CIGM90.856	AABBAA	*T. urartu*	*T. turgidum*
72	68.111/RGB-U//WARD/3/ T. URARTU 551) CIGM90.857	AABBAA	*T. urartu*	*T. turgidum*
73	68.111/RGB-U//WARD/3/ T. URARTU (553)CIGM90.858	AABBAA	*T. urartu*	*T. turgidum*
74	68.111/RGB-U//WARD/3/ T. URARTU (554)CIGM90.859	AABBAA	*T. urartu*	*T. turgidum*
75	68.111/RGB-U//WARD/3/ T. URARTU (555)CIGM90.860	AABBAA	*T. urartu*	*T. turgidum*
76	DOY1/ T. URARTU (560) CIGM90.573	AABBAA	*T. urartu*	*T. turgidum*
77	DOY1/ T. URARTU (563) CIGM90.574	AABBAA	*T. urartu*	*T. turgidum*
78	DOY1/ T. URARTU (564) CIGM90.575	AABBAA	*T. urartu*	*T. turgidum*
79	GAN/ T.BOEOTICUM(7) CIGM93.78	AABBAA	*T. boeoticum*	*T. turgidum*

Molecular analysis: The germplasm was evaluated for molecular diversity using 25 SSR primers at Wheat Wide Crosses and Cytogenetics Laboratory, National Agricultural Research Center (NARC), Islamabad. Genomic DNA

was isolated from fresh leaf tissues of seedlings using the cetyltrimethyl ammonium bromide (CTAB) method with some modifications (Murray *et al*., 1980). Quality of DNA was assessed through 1.0% gel electrophoresis and the samples were stored at 4ºC for future studies. PCR reaction mixtures and programmes were followed according to the published data (Roder *et al.,* 1998).

Data analysis: Clusters were constructed through NTSYSpc software (version 2.02a, Applied Biostatistics Inc., New York, NY). Binary (0 or 1) data were generated to construct dendrogram based on the molecular data. The dendrogram with the best fit to a similarity matrix based on the cophenetic (COPH) values using a matrix comparison (MXCOP) program of NTSYS-pc was chosen. Groups and subgroups were determined using arbitrary points of similarity coefficients according to the software programme (Rohlf, 1992).

The Polymorphism Information Content (PIC) value for each SSR marker locus (*i*) was calculated based on the formula reported (Keim*et al.*,1992).PIC(*i*)=1 $-\sum P_{ij}2$, where P_{ij} is the frequency of the *j*th allele of the *i*thSSR locus and summation extends over *n* alleles.

RESULTS

After initial screening, 25 SSR primers were used to screen the germplasm. PCR result of the marker assays is given in Table-2. Genetic diversity analysis of total 79 amphiploids was carried out using 25 SSR primers. The total number of amplified products was 1082 with an average of 43 bands per primer. Maximum number of bands (112) was produced by primer Xgwm 311-2A while minimum (6) were amplified by Xgwm473-2A. Considering amplified alleles, total number was 126 with an average of 5.04 alleles per primer. Number of amplified alleles varied with different primer assays. Xgwm249-2A and Xgwm311-2A primers amplified maximum 9 alleles followed by a single allele by Xgwm637-4A. All the alleles were polymorphic showing 100% polymorphism. PIC value for all primer assays was calculated. Highest PIC value (0.85) was shown by Xgwm382-2A followed by Xgwm 397-4A (0.12). While the average PIC value was 0.52 per primer (Shete *et al*., 2000). The minimum genetic distance showed by genotypes was zero and maximum genetic distance for both was 1. The average similarity matrix for SSR was 7.05 (data not shown).

Table- 2: Analysis of banding pattern generated by SSR primers in wheat amphiploids 2n=6x=42; BBAAAA)

S. No.	Primer	Total loci	Polymorphic loci	% Poly-morphism	Samples amplified	Scorable Bands	Amplification Products	PIC
1	Xgwm10-2A	7	7	100%	58	73	50-150	0.36
2	Xgwm47.1-2A	5	5	100%	31	36	50-200	0.57
3	Xgwm47.2-2A	6	6	100%	18	28	50-200	0.51
4	Xgwm71.1-2A	6	6	100%	34	80	50-150	0.73
5	Xgwm71.2-2A	6	6	100%	27	61	50-150	0.76
6	Xgwm95-2A	3	3	100%	56	62	50-150	0.37
7	Xgwm122-2A	5	5	100%	17	20	50-150	0.43
8	Xgwm249-2A	9	9	100%	50	92	50-200	0.78
9	Xgwm265-2A	4	4	100%	16	16	50-200	0.36
10	Xgwm296-2A	5	5	100%	15	18	50-100	0.65
11	Xgwm311-2A	9	9	100%	46	112	50-250	0.77
12	Xgwm312-2A	6	6	100%	68	85	50-150	0.57
13	Xgwm372-2A	3	3	100%	15	16	50-200	0.28
14	Xgwm382-2A	8	8	100%	25	45	50-200	0.85
15	Xgwm473-2A	1	1	100%	6	6	50-100	0
16	Xgwm515-2A	5	5	100%	44	65	50-150	0.75
17	Xgwm558-2A	6	6	100%	22	32	50-100	0.76
18	Xgwm5-3A	8	8	100%	31	50	50-200	0.7
19	Xgwm30-3A	4	4	100%	12	14	50	0.38
20	Xgwm162-3A	4	4	100%	34	38	50-200	0.47
21	Xgwm391-3A	4	4	100%	9	12	50-300	0.7
22	Xgwm666.2-3A	4	4	100%	25	31	50-150	0.58
23	Xgwm397-4A	2	2	100%	22	24	50	0.12
24	Xgwm601-4A	5	5	100%	42	49	50-100	0.59
25	Xgwm637-4A	1	1	100%	17	17	50	0

The dendrogram based on the SSR data, separated the accessions into three distinct clusters (Fig.1). Cluster A consisted of 20 genotypes. All the genotypes in this cluster were 100 percent similar. Cluster B consisted of 27 genotypes in which genotype 13 and 16 were 98 percent similar while the genotype 42 was highly diverse with an average genetic distance of 96 percent. Highly diverse genotypes in this group were 52 with 5 percent genetic similarity. Sub Cluster C consisted of 32 genotypes

with varying level of genetic similarity. The genotype 50 appeared to be highly diverse and showed the average genetic distance of 84 percent to 3 other genotypes.

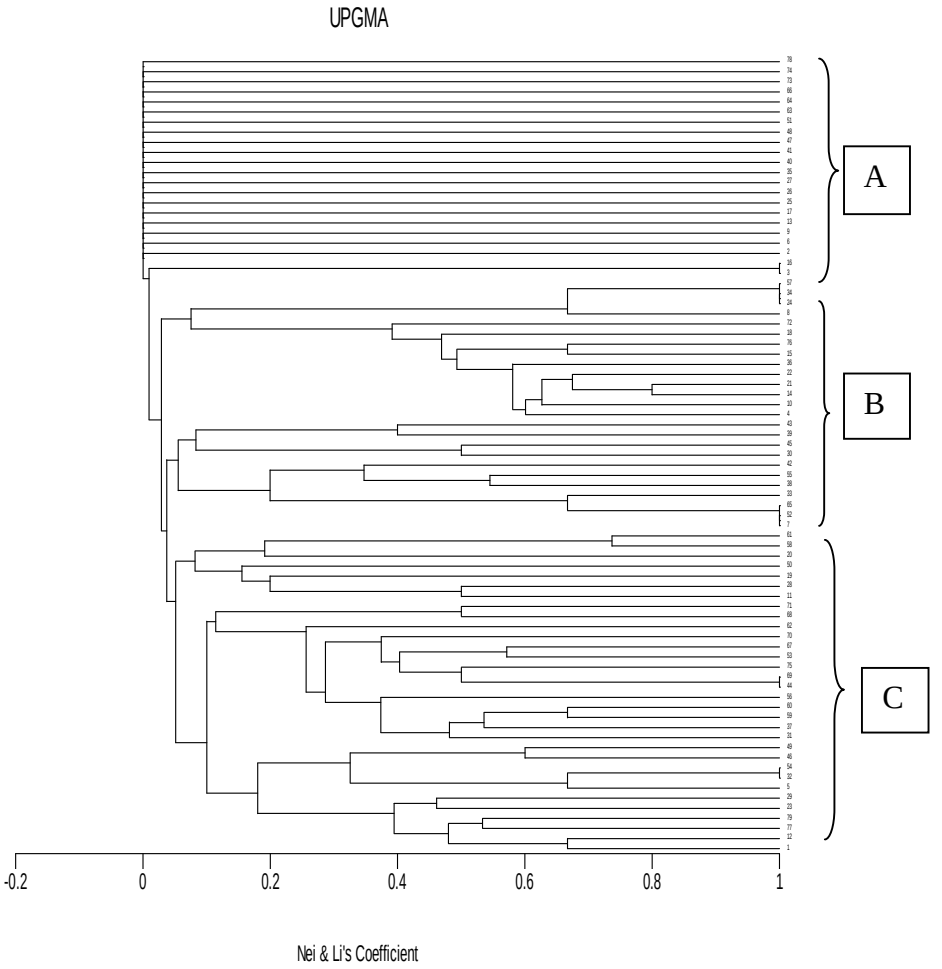

Fig-1: Dendrogram of amphiploids (2n=6x=42; BBAAAA) based on simple sequence repeat (SSR) marker data

DISCUSSIONS

Several decades ago (early 1940's) many traits has been identified for major crops by utilization of wild relatives for supplying genes for improvement of crop that boost production (Plucknett *et al*., 1987). Three decades later this approach obtained great influence and expanded to broad range

of crops (Hoyt, 1988). Further documentary help for the deployment of alien genetic diversity has been of importance over the ancient many decades (Schneider *et al*., 2008). Transfer of alien genes need complex cytogenetic exploitation protocols that assist homologous exchanges. The close wheat progenitors are favored to strengthen the genetic variation of novel genetic reservoirs of wheat crop (Mujeeb-Kazi, 2003; Yao *et al*., 2007). A number of genes controlling different traits of agricultural importance have successfully been transferred from wild wheat progenitors into the hexaploid bread wheat for environmental stresses, numerous pathogens and nutritionally beneficial traits. From wild relatives of wheat, yellow rust resistance genes have been derived and successfully transferred to bread wheat (Riley et al. 1968; Zeller 1973; Kema 1992; Singh *et al*., 1998; Marais *et al.*, 2005; Marais *et al*., 2006; Kuraparthy *et al.*, 2007; Marais *et al*., 2009; Ahmed *et al*., 2013).

There are numerous extensions of A, B and D genome. A and D genome has got tremendous benefit than B within the spectrum due to their closeness to A and D sets existing in cultivated wheat and fall in the order of manipulation for improvement of wheat at a higher rank of desirability. Therefore these primary gene stock sources are main nominee for supplying allelic enrichment. The initial step to initiate alien variation is wide hybridization, comprising both inter-specific and inter-generic hybridization and to shift necessary traits into bread wheat from wild relatives. The policy designed encloses diversity stock covering all gene pools (Mujeeb-Kazi 1995a,b). Wild progenitors of wheat possess abundant unutilized genetic diversity. The A-genome diploid progenitors *T. monococcum, T. urartu* and *T. boeoticum* are notable.

We have explored diversity and molecular variability in 79A-genome amphiploids (BBAAAA) using 25 simple sequence repeat (SSR) primer assays to select the diverse lines for future utilization. The total number of amplification products was 1082 bands with an average 43 bands per primer. All these primers which were utilized showed 100% polymorphism. The average number of polymorphic loci produced by Simple sequence repeat (SSR) was (0.52). The diversity of each primer locus was determined by polymorphism information content (PIC). Higher PIC value (0.85) and polymorphism percentage (100%) generated by these primers demonstrated ted that SSRs are highly efficient for genetic diversity evaluation of synthetic hexaploids and wheat progenitor / ancestors. These primers can also be employed for the selection of superior genotypes for utilization in plant breeding. Furthermore, PCR results based on genetic similarity and clustering revealed accessions 13, 16, 42, 52, and 50 as genetically diverse and can be used for further wheat improvement. In studies conducted in the recent past, SSR primers have been used by several researchers for selection of superior genotypes among

the germplasm and were found as potential tool (Dreisigacker *et al*., 2004; Nicot *et al*., 2005; Danson *et al*., 2006; Rabbani *et al*., 2010).

CONCLUSION

Microsatellite markers utilization possess various benefits like co-dominance, reproducibility, simple analysis of PCR based molecular markers on PAGE, locus specificity, information content and inheritance in Mendelian fashion. Always there is necessity to mobilize genes from wild progenitor to wheat cultivar through hybridisation. Results revealed that 25 microsatellite or SSR primers were used to screen the genetic diversity of total 79 amphiploids. Per primer amplified 43 bands with total 1082 amplified band products were observed. The primer Xgwm311 -2A has produced maximum bands 112 whereas minimum bands 06 were produced by primer Xgwm473-2A. Highest PIC value 0.85 was recorded in Xgwm382-2A primer and lowest PIC value 0.12 in Xgwm397-4A while 0.52 was average PIC value. For the further utilization the PCR based cluster analysis and genetic similarity results revealed that accession 13, 16, 42, 52 and 50 were genetically diverse and recommended for utilization in breeding for future wheat improvement. For wheat improvement, amphiploids proved to be valuable genetic resources against certain abiotic and biotic stresses such as stripe rust where resistance status is used for obtain novel genes through molecular characterization and will supply new diversity to breeders by richness of alleles. Currently, at CIMMYT (International Maize and Wheat Improvement Center) resistant amphiploids are being used to explore novel genes from diploid A Genome and tetraploid *T.turgidum* and the germplasm is an avenue of bread wheat improvement for numerous traits of economic importance.

REFERENCES

Bohn, M., U. H. Friedrich and A.E. Melchinger, Genetic similarities among winter wheat cultivars determined on the basis of RFLPs and SSRs and their use for predicting progeny variance. Crop Sci. **39**(1):228-237(1999).

Cook,J.P., D.M.Witchman, J.M.Martin, P.L. Bruckner and L. E. Talbert, Identification of microsatellite markers associated with a stem solidness locus in wheat. Crop. Sci. **44**:1397-1402 (2004).

Danson, J., M. Lagat, J. Ininda and M. Kimani, Application of simple sequence repeats (SSRs) markers to study the resistance of locally adapted maize hybrids to damaging maize streak virus disease. African Journal of Biotechnology **5**(16): 1430-1434 (2006).

Dreisigacker,S.,P.Zhang,M.L.Warburton, M. Van Ginkel, D. Hoisington, M. Bohn and A. E. Melchinger, SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different mega-environments. Crop Sci. **44**:381–388 (2004).

Figueiredo,E., J.Canhoto and M.Ribeiro, Finger printing and genetic diversity of *Olea europaea* L. ssp. *Europaea*

accessions from the cultivar Galega using RAPD markers. *Sci. Hortic.* **156***:* 24–28(2013).

Gill,B.S.,H.S.Dhaliwal and D.S.Multani, Synthesis and evaluation of *Triticum durum Triticum monococcum* amphiploids. Theor. Appl. Genet. **75**: 912–916 (1988).

Gupta,P.K. and R.K.Varshney, The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica **113**:163–185 (2000).

Gupta, P.K., H.S.Balyan, P.C.Sharma and B. Ramesh, Microsatellites in plants: a new class of molecular markers. Current Science **70**: 45-54 (1996).

Hoyt,E., Conserving the wild relatives of Crops, Rome, Italy, IPGRI/ IUCN/WWF (1988).

Jiang,J., B.Friebe and B.S.Gill, Recent advances in aliengene transfer in wheat.Euphytica **73**:199–212 (1994).

Jefferies,S.P., A.R.Barr, A. Karakousis, J.M.Kretschmer, S. Manning, K.J. Chalmers, J.C. Nelson, A.K.M.R. Islam and P. Langridge, Mapping of chromosome regions conferring boron toxicity in barley (Hordeum vulgre L.). Theor. Appl.Genet. **98**: 1293-1303 (1999).

Keim, P., W. Beavis, J. Schupp and R. Freestone, Evaluation of soybean RFLP marker diversity in adapted germplasm. Theor. Appl. Genet. **85**:205-212 (1992).

Ma,H., R.P.Singh and A.Mujeeb-Kazi, Suppression/expression of resistance to stripe rust in synthetic hexaploids wheat (*Triticum turgidum* x *T. tauschii*). Euphytica **83**:87–93 (1997).

Maric, S., S.Bolaric, J.Martincic, I. Pejic and V.Kozumplink, Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breeding **123**(4): 366-369 (2004).

Mohammadi, S.A. and B.M.Prasanna, Analysis of genetic diversity in crop plants salient statistical tools and considerations: Review and interpretation. Crop Sci. **43**:1235-1248(2003).

Murray,M.G. and W.F.Thompson, Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. **8**: 4321-4325(1980).

Mujeeb-Kazi,A., In: R.J.Singh and P.P. Jauhar (eds), Utilization of genetic resources for bread wheat improvement. CRC series. Taylor and Francis Group, Boca Raton, 61–97 (2006).

Mujeeb-Kazi,A. and G.P.Hettel (eds.), Utilizing Wild Grass Biodiversity in Wheat Improvement: 15 years of Wild Cross Research at CIMMYT. CIMMYT Research Report No. 2. Mexico, D.F (1995).

Mujeeb-Kazi, A. and R.Asiedu, The potential of wide hybridization in wheat improvement. Annals of Biology **11**(1): 1-15 (1995).

Mujeeb-Kazi, A.,Wheat improvement facilitated by novelgenetic diversity and in vitro technology. Plant Tissue Culture **13**:179–210 (2003).

Nicot, N., V. Chiquet, B. Gandon, L. Amilhat. F. Legeai, P. Leroy, M. Bernard, and P. Sourdille, Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). Theor Appl. Genet. **109**:800–805 (2005).

Plucknett, D., N.Smith, J.Williams and N.MurthiAnishetty, Gene banks and the World's Food. Princeton University Press, Princeton, NJ (1987).

Rabbani, M. A., M. S. Masood, Z. K. Shinwari and K. Yamaguchi-Shinozaki, Genetic analysis of basmati and non-basmati Pakistani rice (*Oryza sativa* L.) cultivars using microsatellite markers. Pak. J. Bot. **42**: 2551-2564 (2010).

Röder,M.S., V.Korzun, K.Wendehake, J.Plaschke, M.H.Tixier, P.Leroy and M.W. Ganal, A microsatellite map of wheat. Genetics **149**: 2007– 2023 (1998).

Rohlf,F.J., NTSYS-pc, Numerical taxonomy and multivariate analysis system.Version 1.80. Exeter Soft ware, New York (1992).

Safdar, M., H. Bux**,** A.G. Kazi, A.W. Channa, A.Ali, Z.Akram, A. M. Kazi, Molecular evaluation of D genome based double haploid mapping population of wheat using SSR primers. Pak. J. Biotechnol. **10** (1):27-35 (2013).

Saleh,E.A., M.M.Zaki, A.Rahal, H.M. Sonya, A.M.Bahieldin and A.S.Sadik. DNA fingerprinting of some Herbicide Tolerant Streptomyces species and PCR-isolation of Bar Gene. Pak.J.Biotechnol.**9**(1):39-48 (2012).

Ahmed S., H. Bux, A.Rasheed, A. G. Kazi, A.Rauf, T.Mahmood and A. Mujeeb-Kazi.Stripe rust resistance in *Triticum durum–T. monococcum* and *T. durum – T. urartu* amphiploids. Australasian Plant Pathol.DOI 10.1007/s13313-013-0237-8 (2013)

Schneider, A., I. Molnar and L. M. Molnar, Utilisation of Aegilops (goatgrass) species to widen the genetic diversity of cultivated wheat. Euphytica **163**:1-19 (2008).

Shete.S., H.Tiwari and R.C.Elston, On estimating the heterozygosity and polymorphism information content value. Theoretical Population Biology 57: 265-271 (2000).

Sofalian, O., N. Chaparzadeh, A. Javanmard and M.S.Hejazi, Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. J. Agri. Biol.10:465-468 (2008).

Wilson,A.S., On wheat and rye hybrids. Trans. Proc. Bot. Sco. Edinburgh **12**: 286-288(1876).

Yao,G., J.Zhang, L.Yang, H.Xu, Y. Jiang, L.Xiong, C.Zhang, Z.Zhang, Z.Ma and M.E.Sorrells, Genetic mappingof two powdery mildew resistance genes in einkorn (*Triticum monococcum* L.) accessions. Theoretical and Applied Genetics **114**: 351–358(2007).